Supplementary data:

Supplementary Materials and methods

cDNAs and constructs

AAT-pcDNA3.1 was constructed by performing a PCR on AAT-pcMV6-XL5 using the following primers: forward primer GGT GCT AGC GAA ATG CCG TCT TCT GTC TC containing a NheI site (underlined); and reverse primer ACC TCT AGA CAG TTA TTT TTG GGT GGG ATT C containing an XbaI site (underlined). The PCR product was purified using the Wizard SV Gel and PCR clean-up system (Promega), digested with NheI and XbaI and subcloned into pcDNA3.1. HA (YPYDVPDYA) and Myc (EQKLISEEDL) tags were introduced at the C-terminus of AAT by PCR amplification with Pfu-Turbo polymerase from Invitrogen (Grand Island, NY) using AAT-pCMV6-XL5 as a template. The forward primer GGT AAG CTT GAA ATG CCG TCT TCT GTC TCG TGG G was designed with a HindIII (underlined) site and the reverse primers ACC TCT AGA TTA **GGC ATA ATC AGG CAC ATC ATA AGG ATA** ACC TTT TTG GGT GGG ATT CAC CAC and ACC TCT AGA TTA **CAG ATC CTC CTC AGA AAT CAG CTT TTG CTC** ACC TCC TTT TTG GGT GGG ATT CAC CAC had the sequence encoding for the HA-epitope (in bold) and a Myc-epitope (in bold), respectively. Both reverse primers had the XbaI restriction site (underlined). The PCR products were cut with HindIII and XbaI and inserted into pcDNA3.1 to obtain **HA-AAT-pcDNA3.1** and **Myc-AAT-pcDNA3.1**. The Z mutation was introduced into AAT-pcDNA3.1, HA-AAT-pcDNA 3.1 and Myc-AAT-pcDNA3.1 by site directed mutagenesis with the QuickChange™ site-directed mutagenesis kit (Stratagene) to obtain **ATZ-pcDNA 3.1**, **HA-ATZ-pcDNA 3.1 and Myc-ATZ-pcDNA 3.1** respectively. The

forward primer was GCT GTG CTG ACC ATC CGA C**A**A GAA AGG GAC TGA AGC TGCT GGG and the reverse primer was CCC AGC AGC TTC AGT CCC TTT CT**T** GTC GGA TGG TCA GCA CAG C (point mutation in bold). **ATZ-GFP** was prepared by performing a PCR using Myc-ATZ-pcDNA 3.1 with the forward primer GGT GCT AGC CAT GCC GTC TTC TGT CTC GTG G containing a Nhe I site (underlined) and the reverse primer GAA TTC CAG ATC CTC CTC AGA AA CAG containing an EcoRI (underlined) restriction site. The purified PCR product was digested with NheI and EcoRI and subcloned into pEGFPN2. The cDNA for human wild type Neuroserpin from Origene was subcloned into pcDNA3.1 by excision from neuroserpin pcMV6-XL5 with EcoRI and XbaI and posterior ligation into pcDNA3.1 to obtain **neuroserpin-pcDNA3.1**. The **Portland** mutation was introduced by site-directed mutagenesis using the forward primer GAA AAT ATT CTC TTC TCT CCA TTG AG**A** ATT GCT CTTG CAA TGG GAA TGA T and the reverse primer ATC ATT CCC ATT GCA AGA GCA AT**T** CTC AAT GGA GAG AAG AGA ATA TTT TC (point mutation in bold). Human pro-insulin-pCMV6-XL4 cDNA was subcloned into pcDNA3.1 using the following primers: the forward primer GGT GCT AGC GAA ATG GCC CTG TGG ATG C containing a NheI site (underlined) and the reverse primer containing an XbaI site was ACC TCT AGA CAG TTA TTT TTG GGT GGG ATT C. The PCR product was purified, digested with NheI and XbaI and then ligated into the NheI-XbaI digested pcDNA3.1 to obtain **pro-insulin-pcDNA3.1**. The cDNA for human wild type calnexin was obtained from Origene in the pCMV6-XL5 expression vector. **Calnexin-GFP and calnexin-pcDNA3.1** were constructed by performing a PCR on calnexin-pcMV6-XL6 using the following primers: The forward primer GTT GCT AGC GCC ACC ATG GAA GGG

AAG TGG TTG CT containing a NheI site (underlined) at the N-terminus and the reverse primers containing a BamHI site (underlined) were: AAC GTG GAT CCC CTC TCT TCG TGG CTT TCT GTT and AAC GGA TCC CAC TCT CTT CGT GGC TTT CT respectively. The PCR product was purified, digested with NheI and BamHI and subcloned into EGFPN2 or pcDNA 3.1. All the cDNAs were fully sequenced to ensure that there were no PCR errors.

Supplementary Figures Legends

Supplementary Figure S1. Myc-ATZ, HA-ATZ and ATZ-GFP have similar distribution in Hepa 1-6 cells. A) Hepa 1-6 cells were transiently transfected with HA-ATZ-pcDNA3.1 and Myc-ATZ-pcDNA3.1 and analyzed by confocal fluorescence microscopy at the indicated time points. Cells were co-stained with primary mouse monoclonal antibodies against the HA-tag and the rabbit polyclonal against the Myc-tag at the indicated time points. Secondary staining was carried out using Cy3-conjugated anti-mouse and FITC-conjugated anti-rabbit secondary antibodies. B) Hepa 1-6 cells were transiently transfected with HA-ATZ-pcDNA3.1 and ATZ-GFP and analyzed by confocal fluorescence microscopy at the indicated time points. Cell were co-stained with primary mouse monoclonal antibodies against the HA-tag. Secondary staining was carried out using Cy3-conjugated anti-mouse secondary antibodies.

Supplementary Figure S2. In Huh-7 cells, ATZ colocalizes with calnexin in the ER at the 24 h time-point and to IBs at the 48 h time-point after transfection. Huh-7 transiently

transfected with ATZ-GFP were stained with rabbit polyclonal antibodies against calnexin and Cy3-conjugated anti-rabbit secondary antibody. Lower panel shows a magnified detail (x10) of the images shown above and indicated by the arrowhead; arrow: zones of colocalization of ATZ with calnexin.

Supplementary Figure S3. AAT co-localizes with the Golgi marker GM-130. A) Hepa 1-6 cells were transiently transfected with Myc-AAT-pcDNA3.1 and co-stained with primary mouse monoclonal antibodies against the Myc-tag and the rabbit polyclonal against GM130. Secondary staining was carried out using Cy3-conjugated anti-mouse and FITC-conjugated anti-rabbit secondary antibodies. B) Hepa 1-6 cells were transiently transfected with Myc-ATZ-pcDNA3.1 and co-stained as in A.

Supplementary Figure S4. Immunostaining of calnexin and KDEL in non-transfected Hepa 1-6 cells. Cells were co-stained with primary mouse monoclonal antibodies against KDEL and the rabbit polyclonal antibodies against calnexin. Secondary staining was carried out using Cy3-conjugated anti-mouse and FITC-conjugated anti-rabbit secondary antibodies.

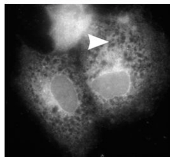
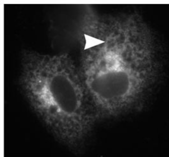
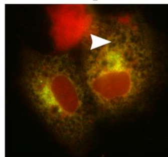
Supplementary Figure S5. ATZ in IBs does not colocalize with the lysosomal marker Lysotracker red. Hepa 1-6, transiently transfected with Myc-ATZ-pcDNA3.1, were incubated with 1 μM Lysotracker Red for 30 min at 37ºC. Cells were fixed and co-stained with the mouse monoclonal anti-Myc antibody and the Cy3-conjugated anti-mouse secondary antibody.

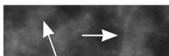
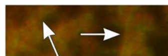
A
Myc-ATZ
HA-ATZ
merge
B
ATZ-GFP
HA-ATZ
merge
24h
48h
24h
48h

Human hepatoma HUH-7

calnexin ATZ-GFP merge

24h

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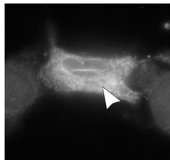
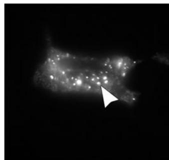
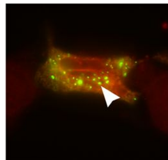
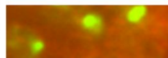
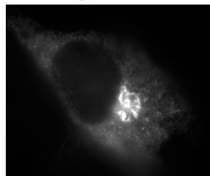




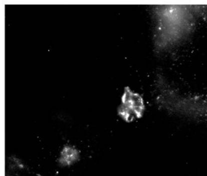


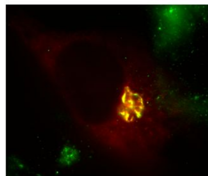
A

Myc-AAT | GM130 | merge

48h



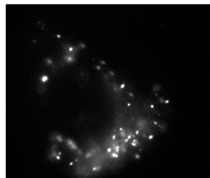


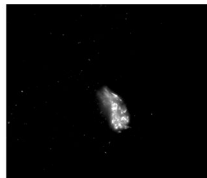


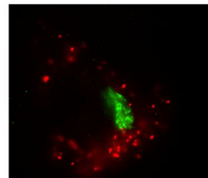
B

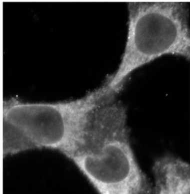
Myc-ATZ | GM130 | merge

48h





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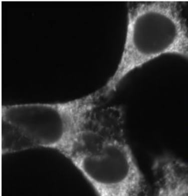
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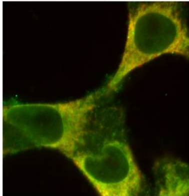
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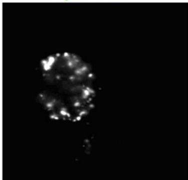
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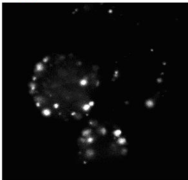
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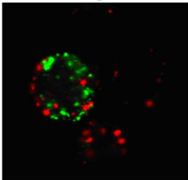
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